



Differential sensitivity to detect prosocial effects of 3,4-methylenedioxymethamphetamine (MDMA) in different social approach paradigms in mice

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Abstract

A recreational drug, 3,4-methylenedioxymethamphetamine (MDMA), has prosocial effects including increased sociability, enhancement of trust feelings, and empathy. Although several methods, such as the social interaction and three chamber tests, have been used, the neural mechanisms underlying the prosocial effects have not been well understood. In the present study, based on a social approach paradigm using a single-chamber apparatus, we have developed two reproducible and simple social approach tests, SAT1 and SAT2, in ICR mice. In the SAT1, an unfamiliar mouse was set in a wire mesh cylinder cage that was placed in the center of a rectangular open field, while in the SAT2, an unfamiliar mouse was set in a wire mesh rectangular cage that was placed along a wall of a rectangular open field. Although MDMA treatment enhanced sociability in both SAT1 and SAT2, the ratio of high sociability mice was higher in the SAT2 than in the SAT1, indicating a differential sensitivity to detect the prosocial effects. Thus, we suggest that the SAT2 is a promising and suitable method to explore the neuronal mechanisms underlying the effects of MDMA.

KEYWORDS

drug abuse, MDMA, mouse, prosocial effect, social approach

1 | INTRODUCTION

The recreational drug, 3,4-methylenedioxymethamphetamine (MDMA), is abused worldwide. MDMA has prosocial effects such as increased sociability, enhancement of trust feelings, and empathy, and the effects are expected to be applicable for the treatment of post-traumatic stress disorder and autism spectrum disorders.^{1,2} However, the neural mechanisms underlying the effects have not

been well understood. Although several methods, including social interaction test, three chamber test, and social conditioned place preference test, have been frequently used³⁻⁶, highly reproducible and simpler experimental methods are important and beneficial for the investigation of the neural mechanisms of prosocial effects of MDMA. In the present study, we have developed two tests based on a social approach paradigm in mice using a single-chamber apparatus and compared the sensitivities of these tests to detect the prosocial effects of MDMA.

Satoko Mukai and Sao Nakada contributed equally to this work.

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2 | METHODS

Male ICR mice (7–10 weeks old, $n = 69$) were bred in-house at Kanazawa University and group-housed at a constant ambient temperature ($22 \pm 2^\circ\text{C}$) under 12-h light/dark cycle (lights on at 08:45), with food and water available ad libitum. MDMA, provided by Dr Tatsunori Iwamura (Matsuyama University, Matsuyama, Japan), was dissolved in saline.

The single-chamber social approach test (SAT) was performed in two ways (SAT1 and SAT2), as previously mentioned, with modifications.^{7–9}

2.1 | SAT1

The schematic diagram of the apparatus and the experimental timeline are shown in Figure 1A,B, respectively. On day 1, each test mouse

was habituated to an open field (59.5 cm long \times 42 cm wide \times 30 cm high) containing an empty wire mesh cylinder cage (10 cm diameter \times 10 cm high) placed at the center of the field for 15 minutes (habituation). Immediately after the habituation, the test mice were briefly removed from the field and an age- and sex-matched unfamiliar ICR mouse was placed into the cage. The test mice were then placed back into the field and allowed to freely explore for 30 minutes (test). These procedures were repeated on day 3, but the test mice received intraperitoneal (i.p.) injection of either saline or MDMA (5 mg/kg) immediately before the test. The dose of MDMA was determined based on previous reports.^{4–6,10} In some mice, the test was also repeated on day 5. The time spent in the “social area” (an 8-cm region surrounding the cage as shown in Figure 1A) during the test on days 1, 3, and 5 was automatically measured using Smart 3.0 software (Panlab Harvard Apparatus, Holliston, MA, USA) when the center of the mouse was located within this area and defined as social approach (SA) time. MDMA-treated test mice that exhibited

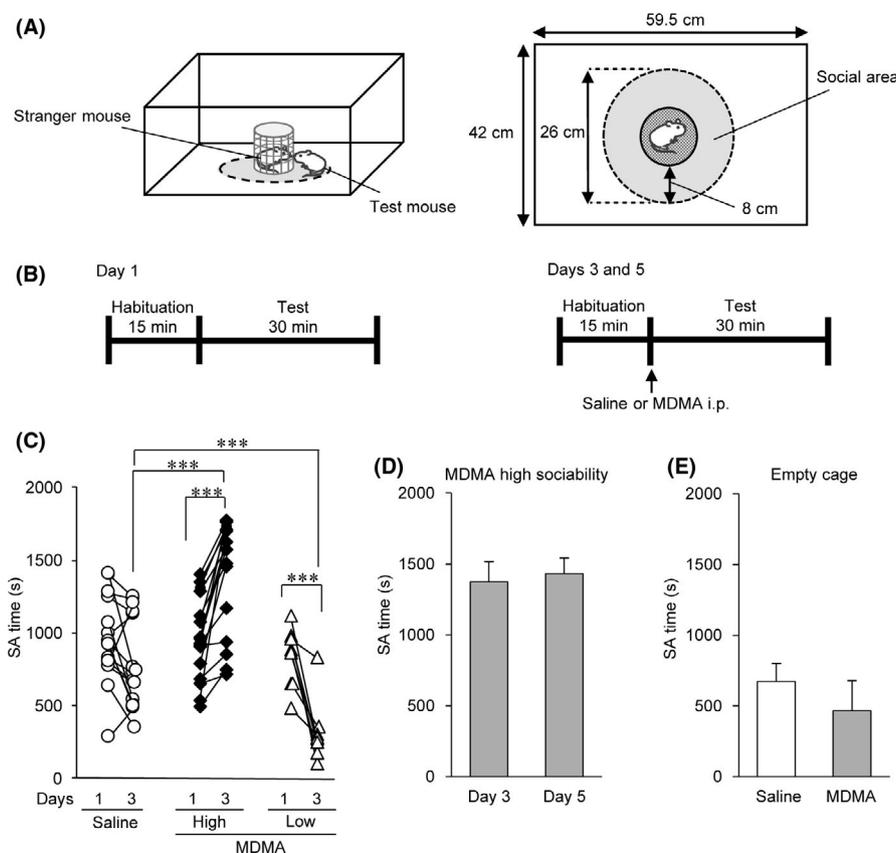


FIGURE 1 The effects of 3,4-methylenedioxymethamphetamine (MDMA) on social approach behavior in the social approach test 1 (SAT1). A, Schematic diagram of the apparatus used in this study. The time spent in the social area (an 8-cm region surrounding the wire mesh cage containing a stranger mouse, SA time) was measured. B, Experimental timeline. SA time was measured twice (days 1 and 3). Mice were administered intraperitoneally with either saline (10 mL/kg) or MDMA (5 mg/kg) immediately before the test on day 3. A subset of the MDMA-treated high sociability mice received a second MDMA administration and were tested again on day 5. C, SA time on days 1 and 3 of saline-treated (saline, day 1, 946 ± 77.6 s vs. day 3, 790 ± 80.7 s, $n = 15$, $P = .128$), MDMA-treated high sociability (day 1, 960 ± 71.1 s vs. day 3, 1406 ± 97.2 s, $n = 16$, $P < .0001$) and low sociability mice (day 1, 848 ± 61.2 s vs. day 3, 296 ± 64.0 s, $n = 10$, $P < .0001$) (interaction, $F_{2,38} = 39.4$, $P < .0001$; treatment, $F_{2,38} = 39.4$, $P < .0001$; day, $F_{1,38} = 3.61$, $P = .0650$, two-way repeated measures ANOVA). D, SA time on days 3 and 5 of MDMA-treated high sociability mice (day 3, 1375 ± 142.6 s vs. day 5, 1432 ± 110.0 s, $n = 7$, $t_6 = 0.709$, $P = .505$, paired t test). E, SA time (saline, 674 ± 127.5 s, $n = 6$ vs. MDMA, 466 ± 213 s, $n = 7$, $t_{11} = 0.803$, $P = .439$, Student's t test) when the wire mesh cage was empty on day 3 of saline- or MDMA-treated mice. Data are expressed as means \pm SEM. *** $P < .001$, Bonferroni's post hoc test

more than 10% increase of averaged increase in SA time, calculated from all MDMA-treated mice from day 1 to day 3, were determined as high sociability mice and the remaining were labeled as low sociability mice.

2.2 | SAT2

The schematic diagram of the apparatus and the experimental timeline are shown in Figure 2A,B, respectively. On day 1, each test mouse was habituated to a testing chamber (39 cm long × 42 cm wide × 30 cm high) that had a square hole (9 × 9 cm) in a wall attached an empty wire mesh rectangular cage for 10 minutes (habituation). Immediately after the habituation, an age- and sex-matched unfamiliar ICR mouse was placed in the cage and the test mice were allowed to freely explore the chamber for 30 minutes (test). On days 2 and 4,

the test mice were injected i.p. with either saline or MDMA (5 mg/kg). Five minutes later, the mice were allowed to freely explore the chamber with the wire mesh cage containing an unfamiliar mouse for 30 minutes. The time spent in the “social area” (8 × 25 cm as shown in Figure 2A) during the test on days 1 and 2 was automatically measured using Smart 3.0 software when the center of the mouse was located within this area. As with the SAT1, MDMA-treated test mice that exhibited more than 10% increase of averaged increase in SA time were defined as high sociability mice, and the remaining were labeled as low sociability mice.

Data were expressed as means ± SEM and analyzed by Student's *t* test, paired *t* test, or two-way repeated measures analysis of variance (ANOVA) followed by Bonferroni's post hoc test using GraphPad Prism 6 (GraphPad software, La Jolla, CA, USA). Raw data of Figures 1C–E and 2C–E are shown in Tables S1–S6. Differences with *P* < .05 were considered statistically significant.

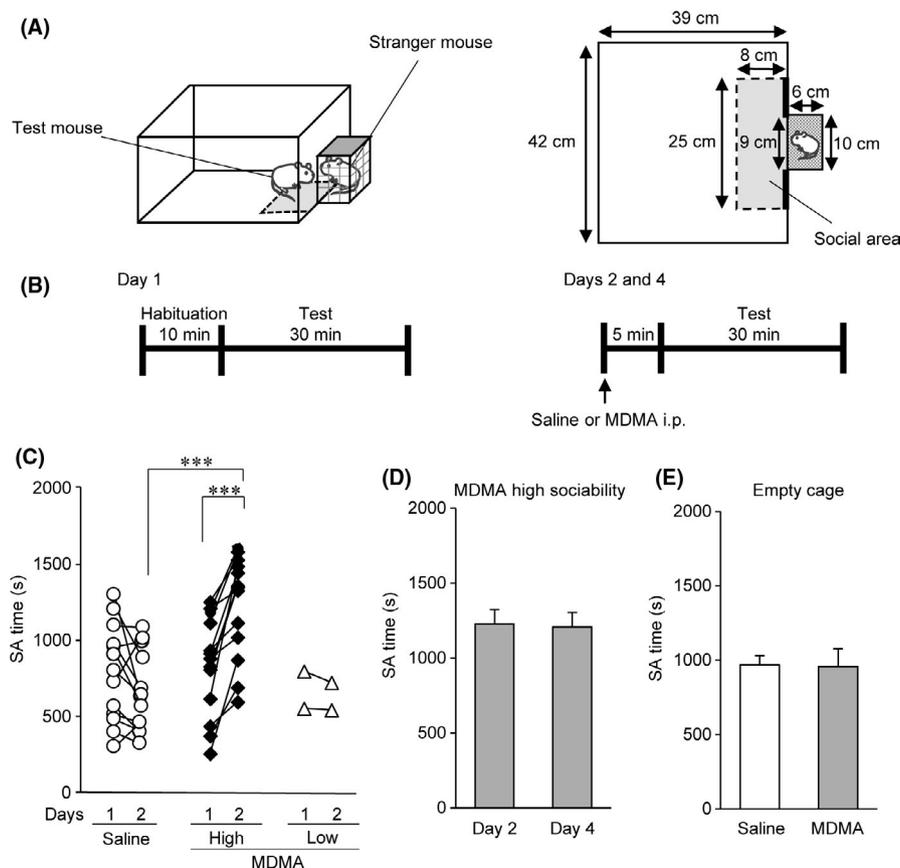


FIGURE 2 The effects of 3,4-methylenedioxymethamphetamine (MDMA) on social approach test 2 (SAT2). A, Schematic diagram of the apparatus used in this study. The time spent in the social area (an 8 × 25 cm area in front of the wire mesh cage containing a stranger mouse, SA time) was measured. B, Experimental timeline. SA time was measured twice (days 1 and 2). Mice were administered intraperitoneally with either saline (10 mL/kg) or MDMA (5 mg/kg) 5 min before the test on day 2. The MDMA-treated high sociability mice received a second MDMA administration and were tested again on day 4. C, SA time on days 1 and 2 of saline-treated (day 1, 785 ± 88.9 s vs. day 2, 658 ± 72.0 s, *n* = 13, *P* = .128), MDMA-treated high sociability (day 1, 832 ± 91.0 s vs. day 2, 1229 ± 93.9 s, *n* = 13, *P* < .0001) and low sociability mice (*n* = 2) (interaction, $F_{1,24} = 32.2$, *P* < .0001; treatment, $F_{1,24} = 7.36$, *P* = .0122; ($F_{1,24} = 8.59$, *P* = .0073, two-way repeated measures ANOVA). D, SA time on days 2 and 4 of MDMA-treated high sociability mice (day 2, 1229 ± 93.9 s vs. day 4, 1208 ± 96.1 s, *n* = 13, $t_{12} = 0.266$, *P* = .795, paired *t* test). E, SA time (saline, 969 ± 60.8 s, *n* = 5 vs. MDMA, 957 ± 119.6 s, *n* = 5, $t_8 = 0.0897$, *P* = .931, Student's *t* test) when the wire mesh cage was empty on day 2 of saline- (*n* = 5) or MDMA-treated mice (*n* = 5). Data are expressed as means ± SEM. ****P* < .001, Bonferroni's post hoc test



3 | RESULTS

In the SAT1, 16 of 26 MDMA-treated mice (61.5%) were high sociability mice and the remaining 10 (38.5%) exhibited low sociability (Figure 1C). The SA time of the high and low sociability mice was significantly longer and shorter on day 3 than day 1, respectively (high sociability, $P < .0001$; low sociability, $P < .0001$, Bonferroni's post hoc test; Figure 1C). While there was no difference in the SA time between day 1 and 3 in saline-treated mice ($P = .128$, Bonferroni's post hoc test; Figure 1C), the SA time on day 1 was not different among saline-treated, high and low sociability groups (saline vs. low sociability, $P > .9999$; saline vs. high sociability, $P > .9999$, Bonferroni's post hoc test). However, high and low sociability mice exhibited longer ($P < .0001$, Bonferroni's post hoc test) and shorter ($P = .0004$, Bonferroni's post hoc test) SA time, respectively, on day 3 than saline-treated mice (Figure 1C). A subset of high sociability mice received a second MDMA administration on day 5. The SA time on day 5 was comparable to that on day 3 ($P = .505$, paired t test; Figure 1D). Compared with saline treatment, MDMA treatment did not affect the SA time on day 3 when the wire mesh cage was empty ($P = .439$, Student's t test; Figure 1E).

In the SAT2, 13 of 15 MDMA-treated mice (86.7%) were high sociability mice (Figure 2C). Because only two mice (13.3%) exhibited low sociability, these mice were excluded from the following analyses. High sociability mice showed significantly longer SA time on day 2 than day 1 ($P < .0001$, Bonferroni's post hoc test; Figure 2C), while saline-treated mice exhibited no difference in the SA time between day 1 and 2 ($P = .128$, Bonferroni's post hoc test; Figure 2C). Saline-treated and high sociability mice demonstrated similar SA time on day 1 ($P > .9999$, Bonferroni's post hoc test), but high sociability mice exhibited significantly longer SA time on day 2 than saline-treated mice ($P < .0001$, Bonferroni's post hoc test; Figure 2C). High sociability mice on day 2 received a second MDMA administration on day 4. The SA time on day 4 was not significantly different from that on day 2 ($P = .795$, paired t test; Figure 2D). Compared with saline treatment, MDMA treatment did not affect the SA time on day 2 when the wire mesh cage was empty ($P = .931$, Student's t test; Figure 2E).

4 | DISCUSSION

In this study, we developed two simple SATs and found that the sensitivity of these tests to detect the prosocial effects of MDMA is different. The ratio of high sociability mice in the SAT2 was higher than that in the SAT1. Previous studies reported that MDMA not only induces prosocial effects but also could enhance negative moods.¹¹ Thus, low sociability might reflect enhanced negative psychological state such as anxiety. Although handling and habituation processes were introduced, some of the test mice might still feel anxious; this anxiety could be augmented by MDMA. Alternatively, since mice are known to avoid staying at the center area of a chamber due to anxiety, some of the test mice in the SAT1 might have hesitated to approach the cage that was located in the center. On the other hand, because the cage was located along a wall in the SAT2, the anxiogenic effect in the SAT2 is likely to be weaker

than in the SAT1. This is further supported by the findings that the SA time for the empty cage of saline-injected mice in the SAT2 was longer than that in the SAT1 (Figures 1E and 2E). These differences might account for the different ratios of high and low sociability mice between SAT1 and SAT2. Additionally, it should be noted that other differences between SAT1 and SAT2, including the size of the entire device, the boundary distance between the stranger mouse cage and the social area, the timing of drug administration, the time of habituation, and the experimental schedule, could affect the SA time in the SATs.

In both the SAT1 and SAT2, the effects of MDMA are specific to the sociability with other mice because MDMA administration did not increase SA time when the cage was empty. Additionally, the prosocial effects of MDMA were reproducibly observed after the second MDMA injection. These characteristics allow us to predict the possibility that the test mice could be divided into high and low sociability groups after first MDMA injection and then pharmacological examinations could be conducted using high sociability mice in the second MDMA treatment. Thus, because of the higher ratio of the high sociability mice, we consider that the SAT2 is a promising and suitable method to examine the neuronal mechanisms underlying the prosocial effects of MDMA in future studies.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

SM and SN conducted the experiments and analyzed the data. HK and RY conducted the experiments. SD analyzed the data and wrote the manuscript. KK designed the research, analyzed the data and wrote the manuscript.

DATA REPOSITORY

The data that supports the findings of this study are available in the supplementary material of this article.

ANIMAL STUDIES

All experiments were performed with the approval of the Institutional Animal Care and Use Committee at Kanazawa University.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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